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Clinical Vignette

A Novel GNAS1 Mutation, R201G, in McCune–Albright Syndrome

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ACTIVATING MISSENSE mutations of the GNAS1 gene, encoding the alpha subunit of the stimulatory G protein, Gs, have been identified in patients with the McCune–Albright syndrome (MAS, characterized by polyostotic fibrous dysplasia [FD], café au lait skin pigmentation, and endocrine disorders).[1–3] The reduced GTPase activity of the mutated protein leads to overstimulation of adenylyl cyclase.[4] Recent studies on the nature of FD suggest that the excess generation of cAMP resulting from activity of the mutated Gs alpha may represent a common (albeit not necessarily the only) pathogenetic mechanism for the diverse, skeletal, and nonskeletal manifestations of MAS.[5,6] Similar mutations of the GNAS1 gene also occur in non-MAS associated FD of bone.[7–9]

MAS was diagnosed in an 8-year-old male presenting with precocious puberty, facial deformities, and typical café au lait spots with a "coast of Maine" profile. Extensive involvement of the cranial vault was apparent on X-ray examination, and a sample of parietal bone demonstrated changes typical of the sclerotic/pagetoid variant of FD.[10] The craniofacial lesions were apparently progressive and were treated with pamidronate which was reported to reduce bone pain as well as to normalize the levels of serum alkaline phosphatase, osteocalcin, and hydroxyproline. At the age of 13, acromegalic bone changes and growth hormone oversecretion were detected.

Genomic DNA was extracted from a surgical sample of the FD parietal bone that was obtained under an institutionally approved protocol for the use of human tissue in research (National Institutes of Health Protocol #97-DK-0055). Mutation analysis was performed by sequencing the polymerase chain reaction (PCR) amplification product (exon 8) in both directions[6] and by sequencing the PCR product obtained with peptide nucleic acid (PNA) inhibition of the normal allele amplification, a novel highly sensitive method especially suited for mutation analysis of mosaic populations.[9] With both assays, a novel C → G (R201G) mutation was detected (Fig. 1).

With the exception of a single case (of polyostotic FD) in which an R201S mutation was identified previously,[11] R201C and R201H have been the mutations found consistently in MAS patients[5] and in non-MAS FD of bone.[9] Thus, of the predicted missense mutations of codon 201, only R201P and R201L remain undetected to date (although R201L has been observed in an isolated, non-MAS endocrine tumor[12] (Table 1).

The diversity of the amino acids encoded by the missense mutations detected so far (basic, uncharged polar, nonpolar) demonstrates that substitution of the R201 per se, rather than the characteristics of the substituting amino acid, is the critical molecular event leading to reduced GTPase activity. It is thought that R201 is an essential component of the "timing device" that regulates GTPase. Bourne et al.[4] have reported that two of the single-point missense mutations in R201 of Gs alpha (R201C and R201H), as found in human tumors, appear to have reduced $k_{cat\text{-}GTP}$, although the methodology and exact values were not presented. They further reported as a personal communication, the work of A.G. Gilman's laboratory in which replacement of the R201 with mutations requiring more than one base pair changes (Val, Ala, Lys, and Glu) in bacterial recombinant Gs alpha also had reduced $k_{cat\text{-}GTP}$. To our knowledge, the $k_{cat\text{-}GTP}$ activity of the remaining single base pair missense mutations (Gly, Ser, Leu, and Pro) have not been studied. Nevertheless, the loss of the highly basic side chain of arginine (which can also be ADP-

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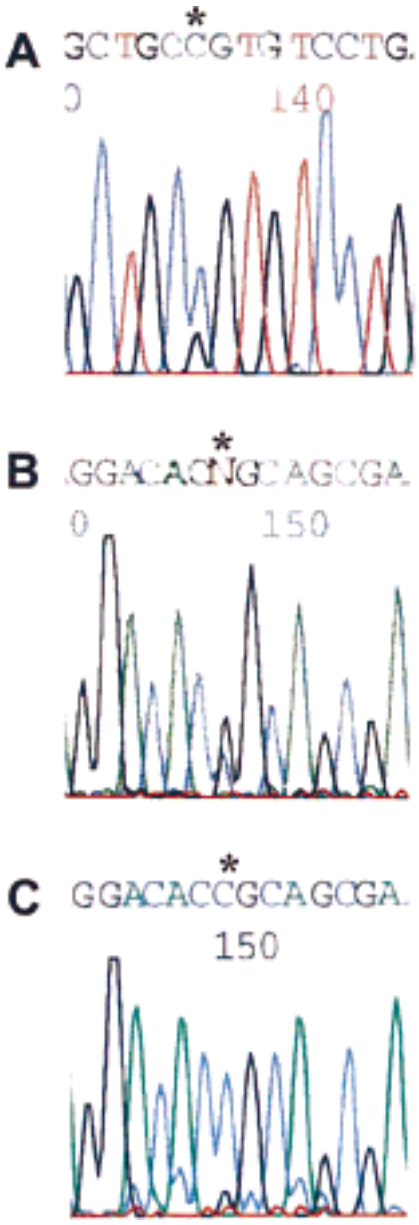

FIG. 1. Demonstration of a novel R201G mutation. Genomic DNA was isolated and analyzed for mutation by two methods using primer sets derived from the specific sequences spanning the R201 region in exon 8 (GenBank accession number X04408.[13] In the first method, gDNA was amplified as previously described.[6] When sequenced in the both directions (A, B), a dual peak (C, G), indicating the heterozygous C → G transition was clearly seen (asterisk). The resulting 5′GGT3′ codon predicts an R201G mutation in the Gs alpha gene product. The mutation was further confirmed by using a novel method[9] whereby a PNA primer, designed to specifically bind to the wild-type sequence, blocks the annealing and extension of the overlapping forward primer. If any R201 mutations are present, the PNA primer does not bind, and the sequence is amplified. In this case (C), sequencing in the reverse direction, the C base was complementary to the mutated G (forward, 5′GGT3′; reverse sequence, 5′AC**C**3′) (asterisk), clearly indicating the presence of the R201G mutation.

TABLE 1. SUMMARY OF PREDICTED AND OBSERVED GS ALPHA R201 MUTATIONS*

Arg (wt)	*Cys*	*Ser*	*Gly*	*His*	*Pro*	*Leu*
5′CGT3′	5′**T**GT3′	5′**A**GT3′	5′**G**GT3′	5′**CA**T3′	5′C**C**T3′	5′C**T**T3′
	MAS		MAS	MAS		
	FD	FD		FD		
	ET	ET		ET		ET

MAS, McCune–Albright Syndrome; FD, fibrous dysplasia, non-MAS; ET, endocrine tumors.

* All substitutions of the third base lead to sense Arg codons.

ribosylated by cholera toxin, a known activator of Gs alpha) at 201 may thus represent the essential change leading to pathological Gs alpha overactivity.

In summary, we have identified a novel R201G mutation of the GNAS1 gene in a patient with all of the clinical features of MAS, adding to the list of known mutations associated with this disease, R201C, R201H, and R201S. Although peculiarities were noted in the phenotype associated with the single reported R201S mutation, obvious major differences in the phenotype that can be correlated to the type of R201 missense mutations have not emerged clearly to date. However, further use of mutation analysis in MAS and FD patients may reveal whether the specific mutation may be a contributing factor to the heterogeneity that is often noted in the lesions of these patients.

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